Spermatogenesis Control

The present invention relates to a pharmaceutical composition for spermatogenesis control and a process for investigating the spermatogenesis as well as a kit usable therefor.

The development of spermia is referred to as spermatogenesis. It is desired to interfere in spermatogenesis if the latter is unbalanced and does not yield functioning spermia. On the other hand, interference in spermatogenesis could also be made use of to carry out a fertility control in male persons.

Therefore, it is the object of the present invention to provide a product by which spermatogenesis can be controlled.

According to the present invention this is achieved by the subject matters defined in the claims.

Therefore, the subject matter of the present invention relates to a pharmaceutical composition adapted to control spermatogenesis. Such a composition comprises:

(a) for positive control
one or more substances of
CREM (cAMP responsive element modulator), a CREM-phosphorylating compound and a CREM expression inducing compound, and/or

(b) for negative control
one or more substances of
a CREM-inhibiting compound, a CREM phosphorylation inhibiting compound, and a CREM expression inhibiting compound.

The present invention is based on the applicant's finding that CREM (cAMP responsive element modulator) is a decisive regulator of spermatogenesis. The applicant has found that CREM is a transcription factor which controls the expression of proteins involved in spermatogenesis. These proteins are referred to as CREM-dependent proteins in the present application. Examples thereof are proacrosin, protamine, Tp-1 (transition protein-1), MCS (mitochondrial capsule seleno protein) and RT7 (mill germ cell specific protein). If there is CREM deficiency, i.e. if CREM is not expressed or expressed only to a reduced extent and not expressed in phosphorylated form, respectively, so that the above proteins are not expressed either or expressed only to a reduced extent, there will be unbalanced spermatogenesis which results in non-functioning spermia.

In a pharmaceutical composition of the present invention, the expression "a CREM-phosphorylated compound" refers to any compounds adapted to phosphorylate CREM, particularly kinases. In addition, the expression "a CREM expression inducing compound" relates to any compounds which can directly or indirectly induce the expression of CREM. Moreover, the expression "a CREM-inhibiting compound" covers any compounds adapted to inhibit CREM, particularly antibodies directed against CREM. Besides, the expression "a CREM phosphorylation inhibiting compound" denotes any compounds adapted to inhibit the phosphorylation of CREM. Such compounds are particularly kinase-inhibitors, such as H7, H8, H89, HA 1004 and Walsh inhibitor. Furthermore, the expression "a CREM expression inhibiting compound" comprises any compounds which can directly or indirectly inhibit the expression of CREM.

The person skilled in the art knows how to determine which substances mentioned for a pharmaceutical composition of the present invention and which amounts thereof are the best for the spermatogenesis control in an individual proband. For example, the following offers itself to the person skilled in the art: preparation of a transgenic mouse which

which expresses an inducible CREB (cyclic AMP responsive element binding protein) mutant, in round spermatids of the testis. This mutant dimerizes with CREM, the mutant being dominant-negative over CREM, i.e. CREM is inhibited by dimerization with dominant-negative CREB. Therefore, the transgenic mouse enables the determination of substances and the amounts thereof, which influence CREM and thus spermatogenesis.

The introduction of a vector containing a promoter enabling the gene expression in round spermatids, such as the protamine promoter (cf. Zambrowicz, B.P. et al., Proc. Natl. Acad. Sci., U.S.A. 90, (1990), 5071-5075) into inseminated oocytes of a mouse, offers itself for the preparation of the transgenic mouse. This promoter controls a DNA which codes for a fusion protein from the mutated CREB and a modified ligand binding domain of the human progesterone receptor (cf. Wang, Y. et al., Proc. Natl. Acad. Sci., U.S.A. 91, (1994), 8180-8184). The mutated CREB does not have serine but alanine at position 133 and thus cannot be phosphorylated, which signifies the loss of its transcription activity. Amino acids 892-933 are lacking in the modified ligand binding domain of the human progesterone receptor, so that this ligand binding domain can no longer be bound by progesterone but only by the ligand RU 486. The latter serves for activating the mutated CREB in the fusion protein.

A process is also provided according to the invention, which is suited to investigate spermatogenesis and control it, respectively. Such a process comprises the determination of CREM and/or CREM-dependent proteins, e.g. proacrosin, protamine, Tp-1, MCS and RT7.

It is possible to use common methods for determining CREM and/or CREM-dependent proteins. It is favorable to determine by means of PCR methods whether the DNA sequences coding for CREM and/or CREM-dependent proteins include mutations. In addition, the possibility presents itself to puncture the

testis to investigate preferably spermatids and more preferably round spermatids of testes and determine the expression of CREM and/or CREM-dependent proteins. For this purpose, CREM and/or CREM-dependent proteins can be determined in a Western blot analysis in which antibodies are used against the individual proteins. The mRNA of CREM and/or CREM-dependent proteins can also be determined in a Northern blot analysis in which DNAs of the individual proteins are used as samples.

A kit is also provided according to the invention, which is suited to determine CREM and/or CREM-dependent proteins. Such a kit comprises:

One or more of (a) to (c)

(a) primers for amplifying DNA coding for CREM and/or CREM-dependent proteins,

(b) antibodies against CREM and/or CREM-dependent proteins, e.g. proacrosin, protamine, Tp-1, MCS and RT7,

(c) DNA samples for mRNA of CREM and/or CREM-dependent proteins, e.g. proacrosin, protamine, Tp-1, MCS and RT7, as well as

(d) standards and detection reagents for one or more of (a) to (c), and

(e) carriers as well as conventional vehicles.

By means of the present invention it is possible to control spermatogenesis, i.e. positively control an unbalanced spermatogenesis, so as to produce functioning spermia and to negatively control normal spermatogenesis thereby inhibiting the formation of spermia. The control of spermatogenesis is reversible, so that the negative control is particularly suitable to control the fertility of a male animal, particularly a male person. By means of the present

invention it is also possible to monitor spermatogenesis, which will be of special importance if controlling interference has been made.